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DOCUMENT-IDENTIFIER: US 6319469 B1
TITLE: Devices and methods for using centripetal acceleration to drive fluid movement in a microfluidics system

Detailed Description Paragraph Right (3):
The invention provides methods and apparatus for the manipulation of samples consisting of fluids, cells and/or particles (generically termed "sample" herein) containing an analyte of interest. The platforms of the invention consist of systems comprising sample input ports, microchannels for fluid flow, reagent reservoirs, mixing chambers, reaction chambers, optical reading chambers, valves for controlling fluid flow between components, temperature control elements, separation channels, electrophoresis channels and electrodes, air outlet ports, sample outlet ports, product outlet ports, mixing means including magnetic, acoustic and mechanical mixers, an on-board power supply such as a battery or electromagnetic generator, liquid and dry reagents, and other components as described herein or known to the skilled artisan. The movement of the sample is facilitated by the judicious incorporation of air holes or air displacement channels that allow air to be displaced but prevent fluid and/or particle loss upon acceleration. Preferably, the disk incorporates microfabricated mechanical, optical, and fluidic control components on platforms made from, for example, plastic, silica, quartz, metal or ceramic. For the purposes of this invention, the term "microfabricated" refers to processes that allow production of these structures on the sub-millimeter scale. These processes include but are not restricted to photolithography, etching, stamping and other means that are familiar to those skilled in the art.

Detailed Description Paragraph Right (48):
Another valve embodiment is a pressure-balanced microvalve, shown in FIG. 10. This type of valve is constructed of three layers of material, comprising two layers of silicon separated by a thin layer of electrically-insulating oxide (i.e., silicon dioxide). A glass layer is bonded onto the top of the valve and advantageously contains inlet and outlet ports. A center plunger fashioned in the middle silicon layer is deflected into a gap contained on the lower silicon layer by application of a voltage between the silicon layers. Alternatively, the plunger is deflected by providing a pneumatic pressure drop into a gap in the lower layer. Irreversible jamming of micromachined parts may be prevented by the application of a thin layer of Cr/Pt to the glass structure. As an electrostatically driven device, this type of valve has many advantages, including that it may be wired directly in the fabrication of the disk. In this embodiment the actuator is a finely tuned device that requires minimal input energy in order to open the valve even at relatively high pressures. These types of valves have been disclosed by Huff et al. (1994, 7.sup.th International Conference on Solid-State Sensors and Actuators, pp. 98-101).

Detailed Description Paragraph Right (73):
Electrochemical detection requires contact between the sensor element and the sample, or between sensor elements and a material such as a gas in equilibrium with the sample. In the case of direct contact between sample and detector, the electrode system is built directly onto the disk, attached to the disk before rotation or moved into contact with the disk after it has stopped rotating. Detectors constructed using a gas vapor to encode information about the sample can be made with the detector external to the disk provided the gas vapor is configured to contact both the sample chamber and the detector. Electrochemical detectors interfaced with the disk include potentiometric, voltammetric and amperimetric devices, and can include any electrochemical transducer compatible with the materials used to construct the microsystem disk.

Detailed Description Paragraph Right (75):
One type of electrochemical detection means useful with the microsystems platforms of the invention is an electrical potential measurement system. Such a system provides a means for characterizing interfacial properties of solutions passed over differently activated flow channels in the instrument. In view of the temperature-controlled nature of the microplatforms of the invention, streaming potentials can also be measured on this device (see Reijenga et al., 1983, J. Chromatogr. 260: 241). To produce streaming potentials, the voltage potential

difference between two platinum leads in contact with a solution at the inner and outer portions of the disk is measured in comparison with a reference electrode. As fluid flows under controlled centripetal motion through the channel, a streaming potential develops in response to fluid interactions with the disk surfaces in a moving field.

Detailed Description Paragraph Right (76):
Alternatively, a platinum electrode is used to generate electroluminescent ions (see Blackburn et al., 37: 1534-9). Chemiluminescence is then detected using one of the optical detectors described above, depending on the wavelength of the chemiluminescent signal. Voltametric components are also useful in microsynthetic platforms of the invention to produce reactive intermediates or products.

Detailed Description Paragraph Right (78):
Electrochemical sensors are also advantageously incorporated into the disk. In one embodiment, an electrochemical detector is provided that uses a redox cycling reaction (see Aoki et al., Rev. Polarogr. 36: 67). This embodiment utilizes an interdigitated microarray electrode within a micromachined chamber containing a species of interest. The potential of one electrode is set at the oxidized potential of the species of interest and the potential of the other electrode is set at the reduction potential of the species of interest. This is accomplished using a dual channel potentiostat, allowing the oxidized and reduced (i.e., redox) chemical state of the sample to be determined, or the chamber may be preset for a particular species. A volume of fluid containing a substance of interest is directed to the chamber. The electrochemically reversible species is then oxidized and reduced by cyclically energizing the electrodes. In this embodiment a molecule is detected by an apparent increase in the redox current. Since non-reversible species do not contribute signal after the first cycle, their overall contribution to the final signal is suppressed. Data analysis software is used to suppress signal due to non-reversible species.

Detailed Description Paragraph Right (79):
In another embodiment, a multichannel electrochemical detector is provided comprising up to 16 lines of an electrode fabricated in a chamber by photolithography with dimensions resulting in each line being 100 .mu.m wide with 50 .mu.m between lines. (see Aoki et al., 1992, Anal. Chem. 62: 2206). In this embodiment, a volume of fluid containing a substance of interest is directed to the chamber. Within the chamber each electrode is set a different potential so that 16 separate channels of electrochemical measurement may be made. Additionally, each electrode potential can be swept stepwise by a function generator. This protocol yields information pertaining to redox potential as well as redox current of the substances. This type of analysis also allows identification of molecules via voltammogram.

Detailed Description Paragraph Right (81):
Another embodiment is a capacitive pressure sensor (see Esashi et al., 1992, Proc. Micro Electro Mechanical Systems 11: 43). In this embodiment, silicon and glass substrates are anodically bonded with hermetically sealed reference cavities. Pressure may be detected by the capacitance change between the silicon diaphragm and an aluminum electrode formed on the glass. A capacitance-to-frequency converter output of a CMOS circuit can be integrated on the silicon substrate or contained in controlling electronics off the disk.

Detailed Description Paragraph Right (84):
Volatile gases on the disk or trapped in the head-space surrounding the disk can be monitored in several ways. For example, a Clark electrode positioned in contact with either the solution of the gases above the disk may be used to detect oxygen content. (Collison et al., 1990, Anal. Chem. 62: 1990).

Detailed Description Paragraph Right (88):
Sample or analyte is collected using means appropriate for the particular sample. Blood, for example, is collected in vacuum tubes in a hospital or laboratory setting, and using a lancet for home or consumer use. Urine can be collected into a sterile container and applied to the disk using conventional liquid-transfer technology. Saliva is preferably applied to the disk diluted with a small volume of a solution of distilled water, mild detergent and sugar flavoring. This solution can be provided as a mouthwash/gargle for detecting antigens, biological secretions and microorganisms. Alternatively, a small sack made of a fishnet polymer material containing the detergent formulation and a chewable resin can be chewed by a user to promote salivation, and then removed from the mouth and saliva recovered and applied conventionally. Amniotic fluid and cerebrospinal fluid are, of necessity, collected using accepted medical techniques by qualified personnel.

Detailed Description Paragraph Right (134):
An illustrative example is immunoassay. While there exist a multiplicity of experimental methodologies for detecting antigen/antibody interactions that are in research and clinical use at the present time, the most robust immunoassay protocols involve "sandwich"-type assays. In such assays, an immobilized antibody is presented to a sample to be tested for the antigenic

analyte specific for the immobilized antibody. A second antibody, specific for a different epitope of the same antigen is subsequently bound, making a "sandwich" of the antigen between the two bound antibodies. In such assays, the second antibody is linked to a detectable moiety, such as a radiolabel or fluorescent label, or a enzymatic or catalytic functionality. For example, horseradish peroxidase or alkaline phosphatase are used to produce a color change in a substrate, the intensity of which is related to the amount of the second antibody bound in the sandwich.

Detailed Description Paragraph Right (136):
In one exemplary embodiment, the reaction chamber comprises an antibody specific for an antigen, where the antibody is immobilized by adsorption of the antibody to the reaction chamber. Contiguous with the reaction chamber is advantageously placed a reagent reservoir containing a second antibody, this antibody being liked to an enzyme such as alkaline phosphate. Sample, which may contain an antigen of interest that is specifically recognized by the above antibodies, is loaded at an inlet port. The disk is spun to first introduce the sample into the reaction chamber containing immobilized antibody, followed by introduction of the second antibody into the reaction chamber after a time sufficient to saturate the immobilized antibody with antigen to the extent the antigen is present in the sample. Alternatively, the sample may be contacted with the second antibody, allowed to interact, then introduced into the reaction chamber. Incubation of the sample with antibody is performed without spinning for about 1 minute. After each incubation, washing buffer from a buffer reservoir is spun into the reaction chamber in order to remove unbound antibody. For alkaline phosphatase assays, solutions of 2 mg/mL o-dianisidine in water, 1 mg/mL B-naphthyl phosphate in 50 mM boric acid/50 mM KCl (pH 9.2) buffer and 100 mM magnesium chloride are delivered to the reaction chamber in the appropriate amounts. The extent of enzyme-linked, secondary antibody binding is evaluated by detection of a purple precipitate using a photodiode or CCD camera.

Detailed Description Paragraph Right (139):
The surface or chamber of the disk for specific binding of the particular cells or cell types of interest is prepared to provide specific binding sites therefor. Typically, an antibody, preferably a monoclonal antibody, is attached to the surface or chamber, wherein the antibody is specific for a cell surface antigen expressed on the cell or cell type of interest. Alteratively, a ligand specific for a cell surface receptor expressed on the particular cell or cell type of interest is used to provide a specific attachment site. Arrays of specifically prepared surfaces or chambers are provided on certain embodiments of the disk. Surfaces and chamber are provided, for example, by contacting the surface with a solution of an appropriate antibody. In the practice of these preparation methods, contact of the surface with the antibody is followed by contacting the surface with a non-specific blocking protein, such as bovine serum albumin. Antibodies and blocking proteins can be contacted with the surface or chamber using a piezoelectrically driven point head (such as are used in ink-jet printing applications) can be advantageously used for this purpose. Alternatively, screen printing, or spraying the antibody solution on the chamber or surface using an airbrush can be employed. These methods are preferred in preparing surfaces and chambers in the 0.1-10 mm scale. In additional alternatives, microlithographic and microstamping techniques can be used to prepare the surface or chamber.

Detailed Description Paragraph Right (153):
In addition, blood gas can be determined using the above device in combination with a disk having integrated electrodes embedded within the hematocrit channel, or having a separate channel devoted to blood gas determination on the hematocrit disk. Blood oxygenation (PO.sub.2) is determined by a Clark-type electrode consisting of a thin Cr--Au cathode and an Ag--AgCl wire anode. The amount of carbon dioxide in the blood is determined by a Severing-type electrode using an ISFET (a type of field effect transistor) as a pH monitor. Blood pH is determined with the use of a Sl.sub.3 N.sub.4 gate ISFET with a reference electrode consisting of a liquid junction and an Ag--AgCl wire electrode. Further examples of such analytical methods for determining blood gases, electrolyte concentration and other information advantageously performed using the hematocrit disk or alternate variations of this disk are described as modifications of the macroscopic-scale methods of Shoji & Esashi (1992, Sensors and Actuators B 8: 205).

Detailed Description Paragraph Right (157):
The activity of enzymes in the protein fraction can be determining using immobilized enzymes (Heineman, 1993, App. Biochem. Biotech. 41: 87-97). For example, blood-specific enzymes (such as glucose oxidase, alkaline phosphatase, and lactate oxidase) can be immobilized in poly (vinyl alcohol OVAL). Lactate oxidase is immobilized on platinized graphite electrodes by sandwiching a thin layer of enzyme between two layers of PVAL. The sensor responds to lactate by the electrochemical oxidation of hydrogen peroxide generated by the enzyme-catalyzed oxidation of lactate that diffuses into the network. The current produced is proportional to the concentration of peroxide, which in turn is proportional to the concentration of lactate. This sensor has been shown to be sensitive to lactate concentrations ranging form 1.7-26 uM.

Detailed Description Paragraph Right (158):
Upon separation, each fraction is interrogated by detection systems to determine the relative components of the fractions. Alternatively, each fraction can be removed from the disk through an outlet port for further study off-device. For example, each fraction can be subjected to simple counting by passing the cells in a thin stream past two electrodes comprising a resistance monitor. As a cell passes through the electrodes a corresponding rise in resistance is monitored and counted. These data are then integrated relative to a standard set of particles distributed according to size to determine the relative number of each cell type in the original sample.

Detailed Description Paragraph Right (190):
To introduce a DNA sample, a valve is opened from an inlet port holding a solution of DNA fragments, or alternatively, the sample is pipetted directly onto the disk. The sample is applied to the separation channel by spinning the disk at 1 to 30,000 rpm, forcing sample and buffer into the buffer filled channel above the gel. Upon introduction of the sample to the separation channel and the sample inlet channel. Sample concentrates at the gel/buffer interface before entering the separation matrix, analogous to sample concentration during conventional slab gel electrophoresis. Electrophoresis is performed at 250 V/cm to effect a separation of DNA fragments, the cathode (positive electrode) being positioned at the outlet end of the channel distal to the sample inlet channel. A laser induced fluorescence detector is positioned at the outlet of the gel filled capillary chamber to detect the labeled DNA fragments, as described above in Example 2.

CLAIMS:

64. An apparatus of claim 62 further comprising a Clarke electrode operatively connected to each of the microchannels of the microsystem platform, wherein each electrode is in contact with a blood sample within a microchannel.

65. An apparatus of claim 62 further comprising a Severing electrode operatively connected to each of the microchannels of the microsystem platform, wherein each electrode is in contact with a blood sample within a microchannel.